[Frontiers in Bioscience 10, 2908-2921, September 1, 2005]

T CELL IMMUNITY IN PATIENTS WITH MALIGNANT GLIOMA: RECENT PROGRESS IN DENDRITIC CELL-BASED IMMUNOTHERAPEUTIC APPROACHES

Yasuharu Akasaki, Keith L. Black and John S. Yu

Maxine Dunitz Neurosurgical Institute, Cedars-Sinai Medical Center, LA, California

TABLE OF CONTENTS

1. ABSTRACT

Despite dramatic advances in surgical technique, imaging, and adjuvant radiotherapy or chemotherapy, the prognosis for patients with malignant glial tumors remains dismal. Based on the current knowledge regarding immune responses in the healthy central nervous system (CNS) and glioma-bearing hosts, we discuss dendritic cell (DC)-based immunotherapeutic approaches for malignant gliomas and the relevance of recent clinical trials and their outcomes. It is now recognized that the CNS is not an immunologically tolerated site, and clearance of arising glioma cells is a routine physiological function of the normal, non-compromised immune system. To escape from immune surveillance, however, clinically apparent gliomas develop complex mechanisms that suppress tumoricidal immune responses. Although the use of DCs for the treatment of glioma patients may be the most appropriate approach, an effective treatment paradigm for these tumors may eventually require the use of several types of treatment. Additionally, given the heterogeneity of this disease process and an immune-refractory tumor cell population, the series use of rational multiple modalities that target different tumor characteristics may be the most effective therapeutic strategy to treat malignant gliomas.

2. INTRODUCTION

Despite dramatic advances in surgical technique, imaging, and adjuvant radiotherapy or chemotherapy, the prognosis for patients with malignant glial tumors remains dismal (1, 2). The median survival rate after diagnosis of glioblastoma multiforme (GBM), the most common and aggressive subtype of malignant glioma, is 12-18 months, with a 2-year survival rate approaching zero (2). The disseminated nature of these neoplasms makes current therapeutic interventions highly ineffective at eradicating all residual intracranial tumor reservoirs. This leads to near universal tumor recurrence, which in turn, contributes to the lethality of this disease.

. The development of a successful treatment modality for malignant gliomas will center on the ability to devise a means of eliminating all intracranial neoplastic foci left behind after surgical resection of the primary tumor mass. This is a daunting task given the highly disseminated nature of the disease process and our current inability to adequately visualize and therapeutically target every remaining tumor cell. Treatment approaches aimed at using the body's own immune system to combat intracranial neoplasms may hold promise for eventually achieving this objective. As demonstrated in numerous experimental

settings (3-6), therapeutic strategies that seek to stimulate immune recognition and clearance of glioma cells have the potential to effect powerful immune-mediated killing of all intracranial neoplastic foci. Many approaches seeking to bolster anti-tumor immunity using a variety of immunotherapies have been tested in rodent brain models. Although many of these strategies have proven highly effective in animals, their translation into human patients has not been as successful. Nevertheless, promising data are presented in the literature, and clinical trials of dendritic cell (DC)-based immunotherapy for patients with malignant brain tumors are currently underway. Based on the current knowledge regarding immune responses in the healthy central nervous system (CNS) and glioma-bearing hosts, we discuss DC-mediated treatment approaches for malignant gliomas and the relevance of recent clinical trials and their outcomes.

3. IMMUNE RESPONSES IN THE CNS

3.1. Immunological privilege of the CNS

The CNS had long been considered an immunologically tolerated site, due to a) the lack of lymphatic drainage, b) the nature of the blood-brain barrier (BBB), in which tight junction between cerebral vascular endothelial cells form a physical barrier to the passage of cells and antibodies, and c) the relative lack of MHC expressions in the healthy organ (7, 8). Despite such a peculiar microenvironment that hinders the operation of the immune system, it has become evident that a variety of leukocyte types, including T cells, B cells, and NK cells, continuously traffic though the CNS as part of normal immune surveillance (9). Most research on leukocyte entry into the CNS from their circulation pools has been directed toward understanding the mechanisms of T cell-mediated inflammation. The mechanisms of immune responses in multiple sclerosis, viral encephalitis, and a number of experimental systems, especially experimental autoimmune encephalomyelitis (EAE) underlie the rationale of the current understanding of CNS immunity (10-14). Based on research into these diseases, activated T cells are allowed to enter the parenchyma of the brain as the first step in the cascade toward CNS inflammation (Figure 1A). When the T cells recognize an antigen, the inflammatory pathway enters a second step (Figure 1B) (15), otherwise T cells leave the brain or undergo apoptosis upon contact with local APCs (Figure 1A) (16, 17). The phenomenon that T cells are inhibited by the anti-proliferative effect of gangliosides (18), TGF-β(19), and other signals (20, 21), may also be relevant to the mechanism of anti-inflammatory responses in the CNS. During the second step, T cells enwrapped in the CNS elaborate chemokines, such as MIP-1α and MCP-1 to recruit various leucocytes into the inflammatory site (Figure 1B) (22, 23). If the T cells succeed in recruiting effective leucocytes such as NK cells, macrophages, and Th1 cells in the third step, CNS inflammation may become clinically apparent (9). When the T cells are eliminated by the signals in the microenvironment before they recruit effective supports, the inflammation may be terminated (Figure 1C). Furthermore, if the recruited leukocytes exhibit tolerogenic responses, the inflammation may also be extinguished (Figure 1C) (14, 24). Thus, it is currently believed that the CNS does not bar the infiltration of peripheral circulating leucocytes but regulates the responses of infiltrating T cells in each of the multiple steps.

3.2. APCs for immune surveillance in the CNS

Primary brain tumors rarely generate extracranial metastasis. Nevertheless, T cell reactivity against tumor associated antigens (TAAs) has been demonstrated in the cervical lymph nodes of brain-tumor-bearing mice (25, 26). The reactivity of TAA-specific T cells is contingent upon effective antigen uptake by APCs. This requires either the presence of resident APC populations or trafficking of exogenous APCs into the brain. Microglia have been recognized as the native APCs and although their presence within malignant gliomas is well established, the antigen presenting function of microglia have been shown to be compromised, possibly due to down-regulation of cell surface MHC expression (27). In this context, it is likely that peripheral APCs that traffic into the CNS are responsible for effective antigen presentation to T cells. The perivascular monocytic cells are thought to arrive from a bone marrow source and accumulate signals from the microenvironment to become mature APCs (8, 9). Schneider *et al.* described the presence of numerous $CD11c^+$ macrophages that strongly expressed HLA-DR and were morphologically distinct from microglia within multiple human glioma specimens (28). Fischer *et al.* demonstrated that functionally maturated $CD11c^+$ myeloid DCs appear and expand at perivascular and intraparenchymatic inflammatory sites concomitant to resident microglia proliferation (29). Furthermore, Yang *et al.* demonstrated the presence of OX-62 expressing DC populations within experimental rodent glioblastomas, strongly suggesting a role for endogenous tumor-infiltrating DCs as APCs in glioma (30). Based on these results, it is likely that peripherally circulating precursors of DCs enwrapped in the perivascular area have an ability to differentiate into mature DCs in the brain parenchyma. This differentiation may be caused by various microenvironmental signals. These exogenous DCs may participate in the maintenance of CNS immune response concomitant with resident APCs.

4. IMMUNITY IN PATIENTS WITH MALIGNANT GLIOMA

4.1. Suppressive effect of malignant gliomas on the immune system

It has recently been identified that glioma cells express various TAAs (31-38), which CTLs can recognize (Table 1). Given the already described ability of the immune system to generate antigen-specific T cell responses in the non-compromised CNS, TAA-expressing gliomas may be eliminated prior to becoming clinically apparent (Figure 2A). Despite this hypothesis, TAA expression was frequently observed on tumor tissues and primary cultured tumor cells established from surgical specimens of glioma patients (32, 38), indicating that these tumor cells have the potential to escape immune surveillance. Although it has been described that clinically apparent cancers are equipped to suppress host T cell immunity (39), it is more plausible that immunodeficient

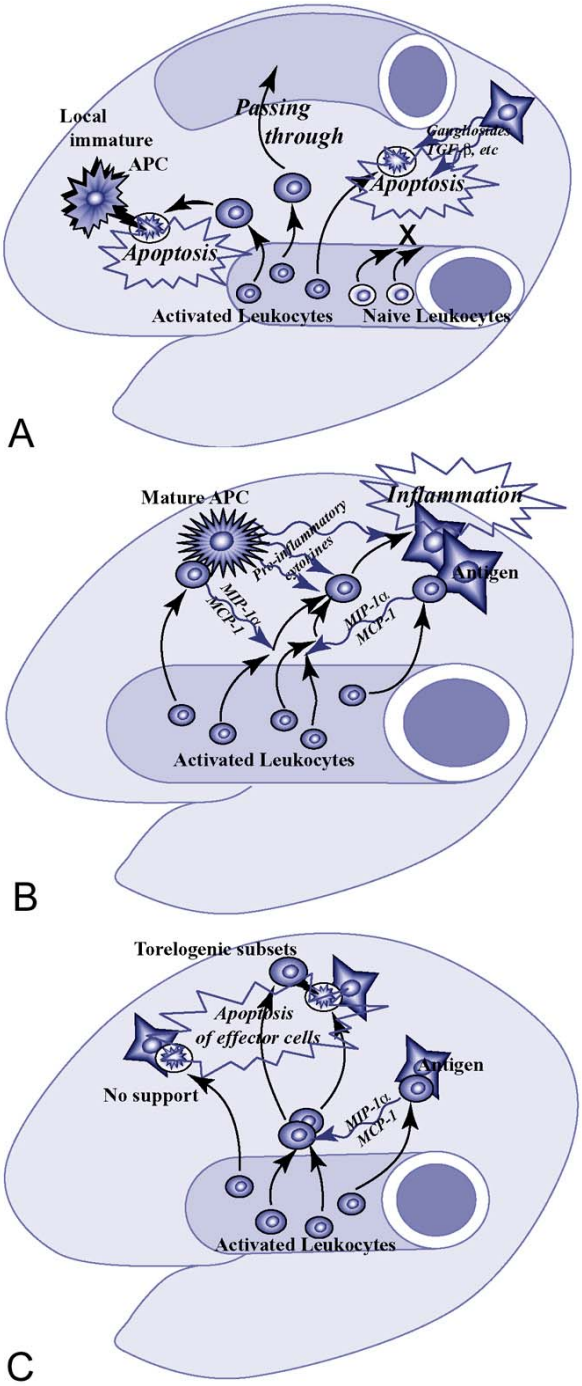

Figure 1 *Schema of immune responses in the CNS* (A) Activated leukocytes are allowed to enter the parenchyma of the brain as the first step in the cascade toward CNS inflammation. When the leukocytes do not recognize any antigen, they leave the brain or undergo apoptosis upon contact with local APCs. The phenomenon that leukocytes are inhibited by the anti-proliferative effect of gangliosides, TGF-β, and other signals may also be relevant to the mechanism of anti-inflammatory responses in the CNS. (B) When the leukocytes recognize an antigen, the inflammatory pathway enters a second step. During the second step, leukocytes enwrapped in the CNS elaborate chemokines, such as MIP-1α and MCP-1 to recruit various leucocytes into the inflammatory site. (C) When the leukocytes are eliminated by the signals in the microenvironment before they recruit effective supports, the inflammation may be terminated. Furthermore, if the recruited leukocytes exhibit tolerogenic responses, the inflammation may also be extinguished.

Table 1. CTL-recognizing TAAs in glioma cells

Antigens	HLA-restriction	Epitope Sequence	Detected Samples
MAGE-1	A1	EADPTGHSY	U-87, U-118, U-373, U-138, IR-801, IR-802, IR-803, Primary glioma cells
AIM-2 (Non-spliced)	A1	RSDSGQQARY	U-87, U-118, U-373, U-138, IR-802, Normal brain tissue, Primary glioma cells
AIM-2 (Spliced)	A1	Not shown	U-87, U-118, U-373, U-138, IR-802, Normal brain tissue, Primary glioma cells
gp100	A2	ITDQVPFSV	U-87, U-138, IR-801, IR-803, Normal brain tissue, Primary glioma cells
HER-2	A2	KIFGSLAFL	U-87, U-118, IR-801, IR-802, IR-803, Normal brain tissue, Primary glioma cells
TRP-2	A2	SVYDFFVWL	Primary glioma cells
GALT-3	A2	TIMAFRWVT IMSRDLVPRI NLLKVNIHI	B2-17, U-251, KINGS-1, T98-G, KALS-1, KNS60, KNS81, ONS76, Normal brain tissue
ARF4L	A2	FLPHFQALHV ALHVVVIGL CITFQVWDV	SF-126, B2-17, U-251, KINGS-1, T98-G, KALS-1, KNS60, KNS81, Normal brain tissue,
IL-13Rα2	A2	WLPFGFILI	U-251, SNB19, T98-G, Normal brain tissue, Primary glioma cells
SART-1	A24	EYRGFTQDF	KNS-81, KALS-1, Normal brain tissue, Primary glioma cells
SART-3	A24	Not shown	Glioma cell lines, Normal brain tissue, Primary glioma cells

individuals display a dramatic increase in cancer incidence. In this regard, it has been shown that gliomas have a higher incidence in immunodeficient states, such as HIV infection and post-transplant administration of immunosuppressive drugs (40-42). Tacconi *et al* reported that the frequency of gliomas in patients with AIDS-related focal mass lesions was approximately 6% (40). These findings strongly suggest the significance of T cell immunity for the immune system's ability to eliminate arising glioma. Although it has already been shown that a variety of glioma-associated mediators induce the host cellular immune suppression (43, 44), these mechanisms are not fully understood. Glioma cells that successfully evade the initial immune killing are able to propagate into established tumors. As they proliferate, these tumor cells accumulate additional mutations, which may confer additional immuno-evasive potentials involving the ability to: a) suppress endogenous APC function (45, 46): b) inactivate tumoricidal CTL responses (47, 48): and c) induce tolerogenic $CD4^+$ T cell response (49). Unfortunately, when the glioma acquires one or more capabilities to suppress tumoricidal immune responses, tumor cells can survive and develop into a clinically apparent tumor (Figure 2B) (39).

4.2. Endogenous APC function in patients with malignant glioma

It has already been shown that both circulating and intratumorally infiltrating DCs are functionally impaired in tumor-bearing animals, and in cancer patients (50-53). In phase I clinical trials of DC-based immunotherapy for glioma patients, it has also been determined that defects in endogenous antigen presentation underlies the impaired cellular immunity seen in glioma patients (54-56). In this context, *in vitro* experiments on human gliomas revealed their ability to alter the function of peripheral blood mononuclear cell (PBMC)-derived DCs (46, 57). Specifically, DCs were cultured with glioma culture supernatants, and this insult resulted in reduced expression of MHC class II, CD80, and CD86 on the DCs. These DCs also impaired IL-12 production and instead increased IL-10 production (46). Although the identification of glioma-associated mediators for these suppressive effects was not determined in these experiments, these results indicate the possibility that endogenous APCs serve as an immune suppressive mediator between glioma-associated factors and T cell responses in glioma patients (Figure 2B).

4.3. Inactivation of tumoricidal CTL responses in the tumor microenvironment

It has long been believed that glioma-associated factors that inactivate tumoricidal CTL responses are a principal reason why gliomas escape immune surveillance. Expressions of Fas ligand (58), B7-H1 (59), and HLA-G (60), have been found to be immune suppressive factors on the cell surface of gliomas, where they probably contribute to inactivation of T cells through direct interaction. On the other hand, CTL inactivation is thought to be relevant to decreased expression of IL-2, IL-12, IFN-γ, and TNF-α, as well as increased expression of IL-4, IL-5, IL-6, IL-10, and TGF-β(47, 48, 61). In recent reports, it has been described that these alterations of cytokine profiles have been observed on tumor-exposed APCs in the tumor microenvironment as well as on tumor cells (46, 49). In the mechanism of CNS immune surveillance, TAA-recognition by the tumor-infiltrating CTLs is followed by the recruitment of tumoricidal supports. If those CTLs fail to recruit effective mediators such as IL-2, IL-12, IFN-γ, and TNF-α, they may be eliminated by various signals from the gliomas and/or glioma-exposed APCs, and then tumor cells can survive to be a clinically apparent glioma. Thus, TAA-specific CTL responses are regulated by not only factors produced by gliomas but also by endogenous APCs that may be altered functionally by glioma-associated mediators in the tumor microenvironment (Figure 2B).

4.4. Tolerogenic $CD4^+$ T cell response in the glioma patients

Although the involvement of tolerogenic T cell responses may be of particular relevance in the setting of cancers (62), the mechanism of their induction in cancer patients remains unclear. Several tolerogenic T cell subsets have been identified within $CD4^+$ cell populations (Table 2). Tr1 is characterized by secretion of IL-10 and TGF-β with negligible production of IL-4 (63, 64). Th3 is also characterized by secretion of IL-10 and TGF-β but differ from Tr1 in their dependence on IL-4 for functional differentiation (65, 66). In addition, a separate category of suppressor $CD4^+$ T cells, identifiable by their co-expression of CD25, has also been described (67). The interrelationship of these regulatory subsets remains unclear, although they have long been known to induce peripheral T cell tolerance caused by the suppression of activated Th1 and Th2 cells (64), as well as by induction of

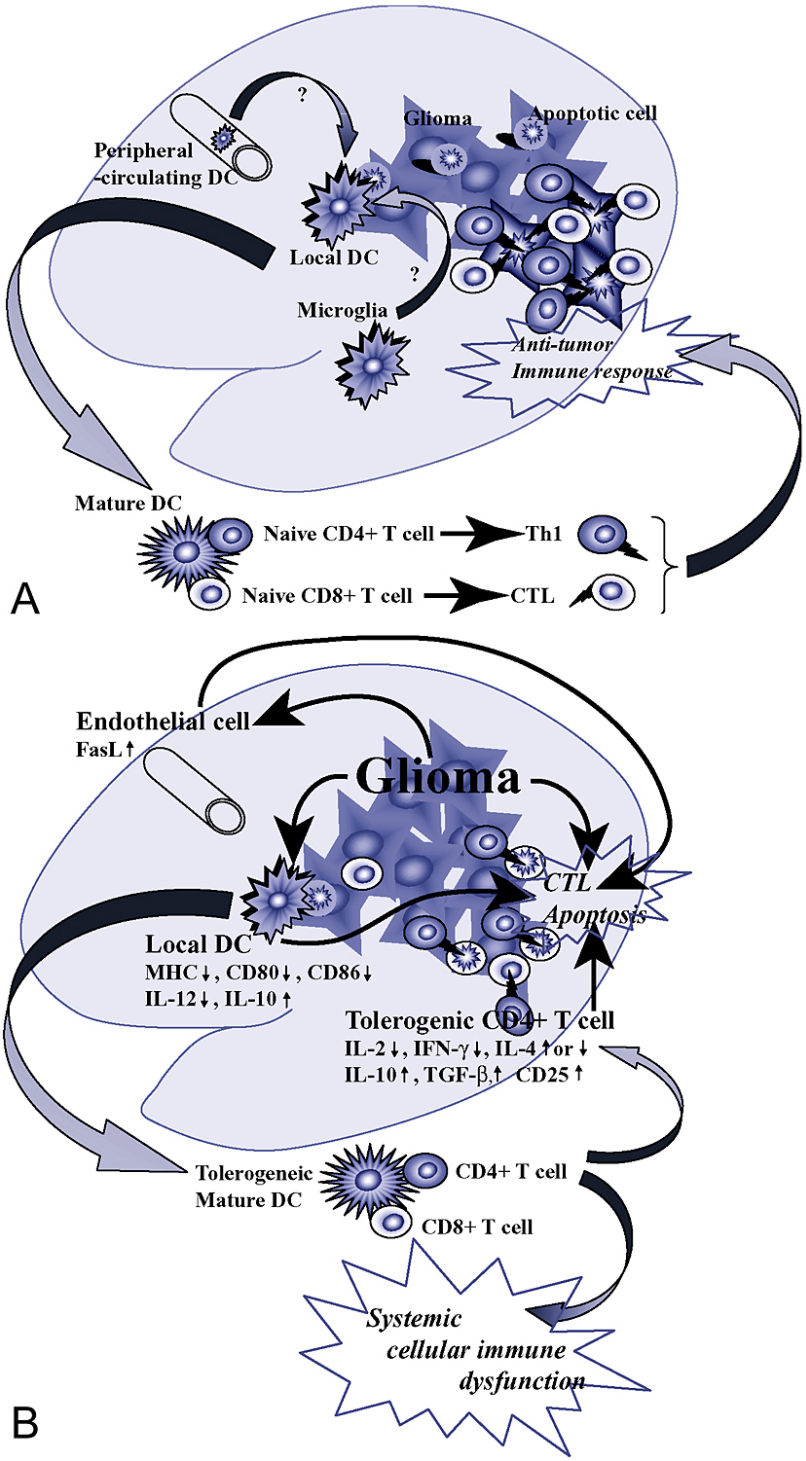

Figure 2. *Schema of immune surveillance and escape mechanism on glioma* **(A)** The reactivity of TAA-specific T cells against glioma is contingent upon effective antigen uptake by APCs in the brain. This requires either the presence of resident APC (microglia) populations or trafficking of exogenous APCs (peripheral DCs) into the brain. These brain DCs may participate in the maintenance of CNS immune response by generating TAA-specific $CD4^+$ and $CD8^+$ T cell responses. **(B)** As glioma cells proliferate, they accumulate mutations, which may confer immuno-evasive potential. Gliomas create a complex microenvironment to escape from immune surveillance, in which they probably produce various factors to alter the host's immune response against tumor cells, as well as to suppress tumoricidal CTL responses through direct effect.

Table 2. Cytokine profile of $CD4^+$ T cell subsets

	$CD4^+$ T cell subsets					
Cytokine	Th0	Th1	Th2	Th3[1]	Tr1[1]	$CD25^+$ T cells[1]
IFN-γ	++	+++	+/-	+/-	+	?
IL-4	++	+/-	+++	++	-	++
IL-10	+	+	+	+++	+++	++
TGF-β	++	+	++	+++	+++	++

[1] refers to the tolerogenic subsets

apoptosis in circulating pools of activated CTLs (68). It is now recognized that apart from generating anti-tumor immune responses, DCs are also capable of initiating tolerogenic $CD4^+$ T cell responses to inciting antigens (69, 70). In the absence of inflammatory signals, antigen-presenting DCs remain immature while entering the regional lymph nodes, and those immature DCs induce tolerance by deletion of antigen-specific T cell clones (71, 72). On the other hand, it has been shown that antigen-processing mature DCs can also induce antigen-specific tolerance, which is mediated by antigen-specific T regulatory subsets (68, 73). In this context, the influence of prostaglandin E_2 (PGE_2) on DCs has been linked to inhibited T cell responses (74). The overexpression of COX-2, the key enzyme governing synthesis of PGE_2 from arachidonic acid (75), has also been well characterized in malignant gliomas (76, 77). These findings indicate that defective antigen presentation in the setting of glioma may be linked to the effect glioma-expressed mediators have on DCs, which could skew the immune response away from tumoricidal subsets to a tolerogenic regulatory phenotype. In this regard, we have recently reported a mechanism of Tr1 induction by mature DCs that phagocytose COX-2 overexpressing glioma (49). In this report, we observed that glioma-exposed mature DCs expressed high levels of IL-10, while their IL-12 production was significantly impaired. These DCs induced a Tr1 response. Selective COX-2 inhibition in COX-2 overexpressing glioma at the time of phagocytic uptake by DCs abrogated this regulatory response and instead elicited Th1 activity. The effect of COX-2 inhibition in COX-2 overexpressing glioma is reversible after administration of PGE_2, indicating that glioma-associated PGE_2 altered DC function to induce a Tr1 response. This tolerogenic Tr1 response probably exerts prominent regulatory effects against anti-tumor immune responses in the CNS, and may have a particular relevance to the observation that cellular immune function is frequently depressed at both the local intratumoral and systemic levels in glioma patients (Figure 2B) (78).

5. PRE-CLINICAL STUDIES OF DC-BASED IMMUNOTHERAPY FOR GLIOMAS

5.1. Experimental DC-based immunotherapy for brain tumors

Substantial evidence has accumulated that implies that CTLs are crucial to the generation of effective anti-tumor immune responses (79, 80). This recognition spurred the search for treatment modalities that could boost tumor-directed T cell responses. Although nonspecific cytokine therapy is capable of supporting and enhancing T cell activity (81), therapeutic strategies to generate TAA-specific immunity from naïve T cells are more promising for researchers seeking to induce effective anti-tumor responses (82). Early vaccination strategies for glioma relied on subcutaneous inoculation of growth-arrested whole tumor cells. Although promising data has been obtained from tumor-cell vaccines (83, 84), the main criticism of these vaccines is that they rely on the inherent antigen presenting capacity of glioma cells, which is poor (85, 86).

There is now considerable evidence to support the contention that the use of professional APCs to initiate tumor-specific T cell responses is a more promising strategy for developing a glioma vaccine. In particular, therapeutic strategies involving DCs, characterized by the presence of large numbers of APC molecules such as MHC class I & II, CD80, and CD86, have received increasing attention (82, 87), and the ability of DCs to generate anti-tumor immune responses in the CNS has been documented (88-93). Most of these experiments have involved *in vitro* isolation of DCs, followed by loading of the DCs with TAAs and injection of the TAA-bearing DCs into syngeneic animals as a glioma vaccine. Bone-marrow-derived DCs pulsed with tumor lysate (88, 93), tumor cDNA (89, 92), apoptotic tumor cells (91), and DCs fused with tumor cells (90), have all been demonstrated to generate tumor-specific immune responses and anti-tumor activity in the brain. In particular, effector functions of $CD8^+$ and $CD4^+$ T cells have been shown to recognize and kill tumor cells. In experiments of DC-based immunotherapy, antigen-exposed DCs are expected to express multiple TAA-derived epitopes in combination with MHC Class I and II molecules on their cell surface. Consequently, this vaccine has the potential to induce immune responses against unidentified multiple TAAs. Moreover, DC-based immunotherapy may maintain protective immunological memory against subsequent tumor challenges (94). These encouraging results led to clinical trials that are currently underway.

5.2. Therapeutic strategies to induce tumor-specific Th1 response

Although traditionally $CD8^+$ CTLs have been the focus of cancer immunotherapy, numerous studies have demonstrated that $CD4^+$ T cells make a critical contribution to the development of effective anti-tumor immune responses (95, 96). $CD4^+$ T cell activity helps create effective anti-tumor immunity, which is believed to be performed mainly by an antigen-specific Th1 subset. This subset occasionally interacts with resident APCs to activate them in the tumor microenvironment. This interaction may confer upon Th1 and resident APCs an ability to activate cellular immune responses (Figure 3A, B). Specifically, upon interaction between antigen-specific Th1 and DCs, which is mediated by the adhesion of TCR/CD4 complex to MHC class II, cellular immune responses are boosted by up-regulation of MHC and costimulatory molecule

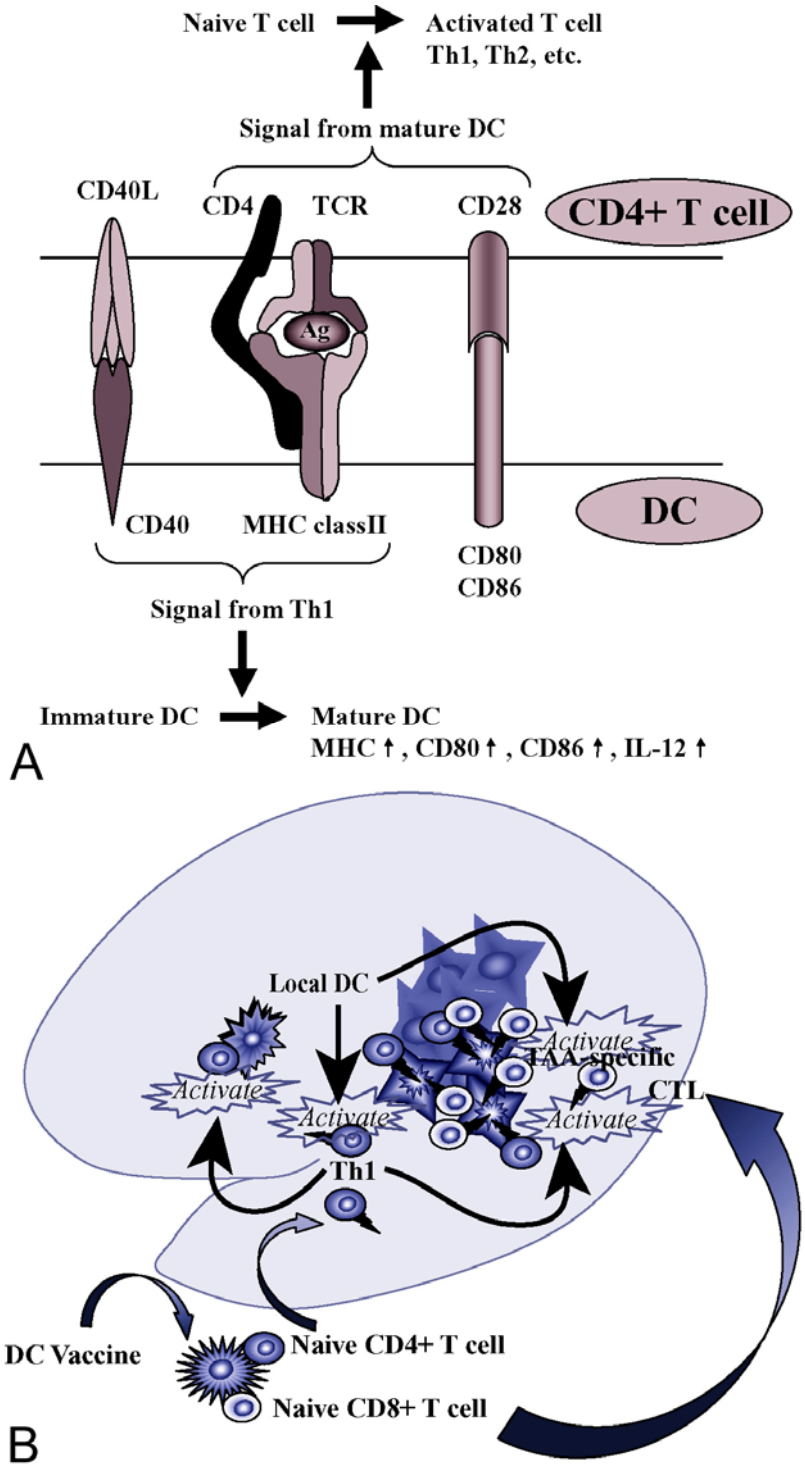

Figure 3. *Interaction of CD4$^+$ T cells with DCs* **(A)** Interaction between Th1 and DCs is mediated by the adhesion of the TCR/CD4 complex to MHC class II. Upon this interaction, cellular immune responses are adamantly boosted by up-regulation of MHC and costimulatory molecule expression, and additional adhesion such as CD40-CD40L and B7-CD28, cytokine signals such as IL-2 and IFN-γ from Th1, and IL-12 from DCs. **(B)** Th1 and resident APCs have the potential to stimulate each other to support TAA-specific CTL responses. The development of a successful anti-glioma vaccine may depend on the helper activity of the antigen-specific Th1 subset, which can interact with resident APCs to activate them in the tumor microenvironment.

expression, and additional adhesion such as CD40-CD40L and B7-CD28, cytokine signals such as IL-2 and IFN-γ from Th1, and IL-12 from DCs (97-99).

$CD4^+$ T cells in patients with malignant tumors, however, frequently polarize away from the Th1 response toward Th2 responses or regulatory subsets (49, 100, 101). Therefore, therapeutic strategies to induce tumor-specific Th1 responses may have a particular relevance to the development of effective anti-tumor immunotherapy. In this regard, IL-12 and IL-18 may be ideal tools to enhance Th1 responses (90, 92). IL-12 is known as a heterodimeric cytokine that plays a central role in promoting Th1 responses and cellular immunity (102). IL-18 also stimulates Th1 responses in collaboration with IL-12 (103). Thus, IL-12 and its collaboration with IL-18 can enhance anti-tumor responses that are induced by DC-based immunotherapy. IL-23 has also recently been identified as an effective cytokine that supports cellular immunity by means of enhancing memory Th1 responses (104-107), indicating that it may be an ideal tool for DC-based immunotherapeutic strategies. As part of a strategy to improve DC function to generate effective Th1 responses, Liu *et al.* used RNA interference technology to modulate the function of PBMC-derived DCs (108). They demonstrated that transfection of DCs with IL-10-specific double stranded siRNA resulted in potent suppression of IL-10 production without inducing DC apoptosis or blocking DC maturation, and also increased IL-12 production after their maturation. It has also been shown that these DC alterations were accompanied by an augmented ability of DCs to boost Th1 responses. Given the already demonstrated ability of endogenous IL-10 to ultimately terminate cellular immune responses (109), DCs modified by IL-10 specific siRNA may be a promising device for cases that are resistant to DC-based immunotherapy.

6. CLINICAL TRAILS OF DC-BASED IMMUNOTHERAPY

6.1. Phase I/II clinical Trials of DC-based Immunotherapy

Yu *et al.* described the use of a DC vaccine in a phase I clinical trial involving patients with newly diagnosed high-grade glioma (54). DCs cultured from patients' PBMC were pulsed *ex vivo* with autologous tumor cell surface peptide isolated by means of acid-elution. Nine patients (2 with anaplastic astrocytoma and 7 with GBM) received a series of three intradermal DC vaccinations following surgical resection and external beam radiotherapy. Anti-tumor cytotoxicity, as determinated by exposing PBMC to autologous tumor targets as part of a JAM assay, was detected in 4 patients. Radiologic evidence of disease progression was detected in 4 patients, who then underwent re-operation subsequent to the third DC vaccination. In 2 patients, the harvested tissue demonstrated robust infiltration of $CD8^+$ and $CD45RO^+$ T cells, which was not apparent in the same patients' tumor specimens resected prior to initiation of the vaccination protocol. Additionally, long-term survival in the study group was compared to data from age- and grader-matched controls with similar disease who underwent surgical resection with external beam radiotherapy. The median survival for the study group was 455 days vs 257 days for the control population, indicating that DC vaccination may confer some survival benefit. No destructive autoimmune response was observed in this study. These data indicated that DC-based immunotherapy for malignant glioma patients was safe and effective in stimulating anti-tumor immune responses as assessed by peripheral cytotoxicity assays and intratumoral T cell infiltration.

These encouraging results led to the expansion of this study into phase II trials, which were reported in 2004 (110). In the phase II trial, 14 patients (4 with anaplastic astrocytoma and 10 with GBM) were thrice vaccinated two weeks apart with autologous DCs pulsed with tumor lysate. One or more TAA-specific CTL clones against MAGE-1, pg100, and HER-2 were established in four of nine patients as part of a HLA-restricted tetramer staining assay. The median survival for the study group was 133 weeks vs 30 weeks for the control population, indicating that significant survival benefit was conferred by DC-based immunotherapy.

. As part of a strategy to improve DC-mediated TAA presentation by means of enhancing tumor cell-DC interaction, Kikuchi *et al* used DC-glioma fusion cells in a phase I clinical trial (56). Eight patients (2 with anaplastic astrocytoma, 5 with GBM and 1 with anaplastic ologodendroglioma) were treated with a series of between 3 and 7 peripheral intradermal vaccinations with DC-autologous glioma fusion cells. Although only minor and temporary responses to therapy were detected in neuroimaging examinations of 2 patients who subsequently developed progressive disease, the capability of this vaccination to induce safely a tumor-specific immune response was demonstrated.

Based on the results of a mouse brain tumor model showing that systemic administration of recombinant IL-12 enhanced the anti-tumor effect of this vaccine (90), Kikuchi and colleagues subsequently reported clinical trials of vaccine therapy using DC-glioma fusion cells and recombinant human IL-12 (111). In this trial, 15 patients (7 with anaplastic astrocytoma, 6 with GBM and 2 with anaplastic oligoastrocytoma) who had progression of their tumor despite radiotherapy and/or chemotherapy received vaccine therapy. The vaccination was scheduled to inject fusion cells intradermally close to a cervical lymph node on day 1, and then recombinant IL-12 (30ng/kg) was injected subcutaneously at the same site on days 3 and 7. This cycle was repeated every 2 weeks for 6 weeks, and a second 6 weeks course was repeated beginning 2 to 5 weeks after the last dose of IL-12 during course 1. As part of a ^{51}Cr releasing cytolytic assay using peripheral blood lymphocytes and autologous glioma cells, cytolytic activity significantly increased after treatment in two patients, while that of other patients was almost nonexistent. In one patient, the activity after the treatment was lower than that before the treatment. Many larger tumor cells containing multiple nuclei and wide cytoplasm were observed in pathologic findings of recurrent tumor specimens, and

although more robust $CD8^+$ T cells infiltrated the area of the tumor compared with primary tumors, infiltration of $CD4^+$ T cell subsets was not observed. The results in this trial may indicate the importance of tumor-specific Th1 responses for immunotherapy to be effective. In other words, the limited success of the DC-glioma fusion cell vaccine may have particular relevance to the failure of tumor-specific Th1 induction and/or the existence of tolerogenic $CD4^+$ T cell subsets.

6.2. Intratumoral injection of DCs

Intratumorally injected DCs can acquire and process tumor antigens *in situ*, migrate via lymphoid vessels to regional lymphoid organs, and initiate significant tumor-specific immune responses, even in the CNS (112, 113). It has been reported that DCs have antigen capturing, processing and trafficking abilities only during their immature state (114). In this context, Yamanaka and colleagues reported the results of a phase I/II clinical trial of DC-based immunotherapy for glioma patients in which 5 patients (1 with anaplastic astrocytoma and 4 with GBM) received intradermal administration of tumor lysate-pulsed DCs, and 5 patients (1 with glioma, 1 with anaplastic mixed glioma and 3 with GBM) received intratumoral administration of immature DCs with intradermal administration of tumor lysate-pulsed DCs (115). In their report, shrinkage of contrast-enhanced lesions was observed in neuro-images in the intradermal plus intratumoral administration group, indicating that intratumorally injected immature DCs may have the potential to induce an anti-tumor immune response by capturing and processing *in situ* TAAs. Based on this result, intratumoral injection of DCs may be a promising strategy for recurrent glioma patients or for patients when a surgical tumor specimen is not available, although the effect of glioma cells to suppress endogenous APC function should not be neglected.

6.3. Subsequent chemotherapy

Wheeler *et al* reported that therapeutic DC-based vaccination synergizes with subsequent chemotherapy to elicit tangible clinical benefits for GBM patients (116). In their report, survival and progression times were analyzed retrospectively in 25 vaccinated (13 with and 12 without subsequent chemotherapy) and 13 non-vaccinated GBM patients. Vaccinated patients receiving subsequent chemotherapy exhibited significantly longer times to tumor recurrence after chemotherapy relative to their own previous recurrence times, as well as significantly longer post-chemotherapy recurrence times and survival relative to patients receiving vaccination alone or chemotherapy alone. Patients exhibiting objective (>50%) tumor regression, extremely rare in GBM, were also confined to the group of vaccine with chemotherapy. Given the already described ability of DC-based immunotherapy to induce TAA-specific immune responses and the functional implication of TAAs to generate refractory activity to chemotherapy (117, 118), it is likely that the elimination of the TAA-expressing glioma cell population by the immune response resulted in increased sensitivity to chemotherapeutic drugs in the residual immune-refractory population. Based on this concept, the series use of rational multiple modalities that target different tumor characteristics may be required for the successful treatment of these tumors.

7. CONCLUSIONS

The CNS is not an immunologically tolerated site. It creates a privileged site that regulates the infiltration, activation, and recruitment of peripherally circulating leukocytes. Despite such a peculiar microenvironment that hinders the operation of the immune system, it is now recognized that clearance of transformed glioma cells is a routine physiological function of the normal, non-compromised immune system. Based on the demonstrated ability of DCs to initiate antigen-specific T cell responses and the potential of the CNS to allow activated T cell infiltration, the use of DCs for the treatment of glioma may be the most appropriate method, and the key to the therapeutic success of this strategy is likely to lie in eliciting TAA-specific CTL and Th1 responses. To escape immune surveillance, however, clinically apparent gliomas develop complex mechanisms that suppress tumoricidal immune responses by suppressing endogenous APCs, inactivating TAA-specific CTL responses, and by inducing tolerogenic T cell responses. Given these complex and divergent mechanisms by which glioma avoids tumor-specific immune responses, an effective treatment paradigm for malignant gliomas may eventually require a multifaceted approach combining two or more different therapeutic strategies to enhance anti-tumor responses and to reduce the ability of gliomas to suppress immune responses. Such scenarios may involve the use of cytokine therapy, genetically modulated DCs and pharmacological treatments. Additionally, given the heterogeneity of this disease process and an immune-refractory tumor cell population, the series use of rational multiple modalities that target different tumor characteristics may turn out to be the most effective therapeutic strategies for the treatment of malignant gliomas. In this context, subsequent chemotherapy may be one of the most promising rational strategies.

8. REFERENCES

1. Legler, J. M., L. A. Ries, M. A. Smith, J. L. Warren, E. F. Heineman, R. S. Kaplan and M. S. Linet: Cancer surveillance series (corrected): brain and other central nervous system cancers: recent trends in incidence and mortality. *J Natl Cancer Inst* 91, 1382-1390 (1999)

2. Surawicz, T. S., F. Davis, S. Freels, E. R. Laws, Jr. and H. R. Menck: Brain tumor survival: results from the National Cancer Data Base. *J Neurooncol* 40, 151-160 (1998)

3. Ehtesham, M., K. Samoto, P. Kabos, F. L. Acosta, M. A. Gutierrez, K. L. Black and J. S. Yu: Treatment of intracranial glioma with *in situ* interferon-gamma and tumor necrosis factor-alpha gene transfer. *Cancer Gene Ther* 9, 925-934 (2002)

4. Liau, L. M., K. L. Black, R. M. Prins, S. N. Sykes, P. L. DiPatre, T. F. Cloughesy, D. P. Becker and J. M. Bronstein: Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. *J Neurosurg* 90, 1115-1124 (1999)

5. Liu, Y., M. Ehtesham, K. Samoto, C. J. Wheeler, R. C. Thompson, L. P. Villarreal, K. L. Black and J. S. Yu: *In situ* adenoviral interleukin 12 gene transfer confers potent and long-lasting cytotoxic immunity in glioma. *Cancer Gene Ther* 9, 9-15 (2002)
6. Herrlinger, U., C. M. Kramm, K. M. Johnston, D. N. Louis, D. Finkelstein, G. Reznikoff, G. Dranoff, X. O. Breakefield and J. S. Yu: Vaccination for experimental gliomas using GM-CSF-transduced glioma cells. *Cancer Gene Ther* 4, 345-352 (1997)
7. Cserr, H. F. and P. M. Knopf: Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. *Immunol Today* 13, 507-512 (1992)
8. Becher, B., A. Prat and J. P. Antel: Brain-immune connection: immuno-regulatory properties of CNS-resident cells. *Glia* 29, 293-304 (2000)
9. Hickey, W. F.: Leukocyte traffic in the central nervous system: the participants and their roles. *Semin Immunol* 11, 125-137 (1999)
10. Anderson, A. C., L. B. Nicholson, K. L. Legge, V. Turchin, H. Zaghouani and V. K. Kuchroo: High frequency of autoreactive myelin proteolipid protein-specific T cells in the periphery of naive mice: mechanisms of selection of the self-reactive repertoire. *J Exp Med* 191, 761-770 (2000)
11. Stohlman, S. A. and D. R. Hinton: Viral induced demyelination. *Brain Pathol* 11, 92-106 (2001)
12. Kuchroo, V. K., A. C. Anderson, H. Waldner, M. Munder, E. Bettelli and L. B. Nicholson: T cell response in experimental autoimmune encephalomyelitis (EAE): role of self and cross-reactive antigens in shaping, tuning, and regulating the autopathogenic T cell repertoire. *Annu Rev Immunol* 20, 101-123 (2002)
13. Adorini, L.: Immunotherapeutic approaches in multiple sclerosis. *J Neurol Sci* 223, 13-24 (2004)
14. Yang, J. S., L. Y. Xu, B. G. Xiao, G. Hedlund and H. Link: Laquinimod (ABR-215062) suppresses the development of experimental autoimmune encephalomyelitis, modulates the Th1/Th2 balance and induces the Th3 cytokine TGF-beta in Lewis rats. *J Neuroimmunol* 156, 3-9 (2004)
15. Qing, Z., D. Sewell, M. Sandor and Z. Fabry: Antigen-specific T cell trafficking into the central nervous system. *J Neuroimmunol* 105, 169-178 (2000)
16. Baron, J. L., J. A. Madri, N. H. Ruddle, G. Hashim and C. A. Janeway, Jr.: Surface expression of alpha 4 integrin by CD4 T cells is required for their entry into brain parenchyma. *J Exp Med* 177, 57-68 (1993)
17. Ford, A. L., E. Foulcher, F. A. Lemckert and J. D. Sedgwick: Microglia induce CD4 T lymphocyte final effector function and death. *J Exp Med* 184, 1737-1745 (1996)
18. Irani, D. N.: Brain-derived gangliosides induce cell cycle arrest in a murine T cell line. *J Neuroimmunol* 87, 11-16 (1998)
19. Wilbanks, G. A. and J. W. Streilein: Fluids from immune privileged sites endow macrophages with the capacity to induce antigen-specific immune deviation via a mechanism involving transforming growth factor-beta. *Eur J Immunol* 22, 1031-1036 (1992)
20. Suvannavejh, G. C., M. C. Dal Canto, L. A. Matis and S. D. Miller: Fas-mediated apoptosis in clinical remissions of relapsing experimental autoimmune encephalomyelitis. *J Clin Invest* 105, 223-231 (2000)
21. Bauer, J., M. Bradl, W. F. Hickley, S. Forss-Petter, H. Breitschopf, C. Linington, H. Wekerle and H. Lassmann: T-cell apoptosis in inflammatory brain lesions: destruction of T cells does not depend on antigen recognition. *Am J Pathol* 153, 715-724 (1998)
22. Benveniste, E. N.: Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. *J Mol Med* 75, 165-173 (1997)
23. Merrill, J. E. and S. P. Murphy: Inflammatory events at the blood brain barrier: regulation of adhesion molecules, cytokines, and chemokines by reactive nitrogen and oxygen species. *Brain Behav Immun* 11, 245-263 (1997)
24. Mekala, D. J. and T. L. Geiger: Immunotherapy of autoimmune encephalomyelitis with re-directed CD4+CD25+ T-lymphocytes. *Blood* (2004)
25. Gordon, L. B., S. C. Nolan, H. F. Cserr, P. M. Knopf and C. J. Harling-Berg: Growth of P511 mastocytoma cells in BALB/c mouse brain elicits CTL response without tumor elimination: a new tumor model for regional central nervous system immunity. *J Immunol* 159, 2399-2408 (1997)
26. Walker, P. R., T. Calzascia, V. Schnuriger, N. Scamuffa, P. Saas, N. de Tribolet and P. Y. Dietrich: The brain parenchyma is permissive for full antitumor CTL effector function, even in the absence of CD4 T cells. *J Immunol* 165, 3128-3135 (2000)
27. Graeber, M. B., B. W. Scheithauer and G. W. Kreutzberg: Microglia in brain tumors. *Glia* 40, 252-259 (2002)
28. Schneider, J., F. M. Hofman, M. L. Apuzzo and D. R. Hinton: Cytokines and immunoregulatory molecules in malignant glial neoplasms. *J Neurosurg* 77, 265-273 (1992)
29. Fischer, H. G. and G. Reichmann: Brain dendritic cells and macrophages/microglia in central nervous system inflammation. *J Immunol* 166, 2717-2726 (2001)
30. Yang, T., T. F. Witham, L. Villa, M. Erff, J. Attanucci, S. Watkins, D. Kondziolka, H. Okada, I. F. Pollack and W. H. Chambers: Glioma-associated hyaluronan induces apoptosis in dendritic cells via inducible nitric oxide synthase: implications for the use of dendritic cells for therapy of gliomas. *Cancer Res* 62, 2583-2591 (2002)
31. Imaizumi, T., T. Kuramoto, K. Matsunaga, S. Shichijo, S. Yutani, M. Shigemori, K. Oizumi and K. Itoh: Expression of the tumor-rejection antigen SART1 in brain tumors. *Int J Cancer* 83, 760-764 (1999)
32. Murayama, K., T. Kobayashi, T. Imaizumi, K. Matsunaga, T. Kuramoto, M. Shigemori, S. Shichijo and K. Itoh: Expression of the SART3 tumor-rejection antigen in brain tumors and induction of cytotoxic T lymphocytes by its peptides. *J Immunother* 23, 511-518 (2000)
33. Nonaka, Y., N. Tsuda, S. Shichijo, M. Ito, Y. Maeda, M. Harada, T. Kamura, M. Shigemori and K. Itoh: Recognition of ADP-ribosylation factor 4-like by HLA-A2-restricted and tumor-reactive cytotoxic T lymphocytes from patients with brain tumors. *Tissue Antigens* 60, 319-327 (2002)
34. Tsuda, N., Y. Nonaka, S. Shichijo, A. Yamada, M. Ito, Y. Maeda, M. Harada, T. Kamura and K. Itoh: UDP-Gal: betaGlcNAc beta1, 3-galactosyltransferase, polypeptide 3 (GALT3) is a tumour antigen recognised by HLA-A2-restricted cytotoxic T lymphocytes from patients with brain tumour. *Br J Cancer* 87, 1006-1012 (2002)

35. Okano, F., W. J. Storkus, W. H. Chambers, I. F. Pollack and H. Okada: Identification of a novel HLA-A*0201-restricted, cytotoxic T lymphocyte epitope in a human glioma-associated antigen, interleukin 13 receptor alpha2 chain. *Clin Cancer Res* 8, 2851-2855 (2002)
36. Liu, G., H. T. Khong, C. J. Wheeler, J. S. Yu, K. L. Black and H. Ying: Molecular and functional analysis of tyrosinase-related protein (TRP)-2 as a cytotoxic T lymphocyte target in patients with malignant glioma. *J Immunother* 26, 301-312 (2003)
37. Liu, G., J. S. Yu, G. Zeng, D. Yin, D. Xie, K. L. Black and H. Ying: AIM-2: a novel tumor antigen is expressed and presented by human glioma cells. *J Immunother* 27, 220-226 (2004)
38. Liu, G., H. Ying, G. Zeng, C. J. Wheeler, K. L. Black and J. S. Yu: HER-2, gp100, and MAGE-1 are expressed in human glioblastoma and recognized by cytotoxic T cells. *Cancer Res* 64, 4980-4986 (2004)
39. Pardoll, D.: Does the immune system see tumors as foreign or self? *Annu Rev Immunol* 21, 807-839 (2003)
40. Tacconi, L., S. Stapleton, F. Signorelli and D. G. Thomas: Acquired immune deficiency syndrome (AIDS) and cerebral astrocytoma. *Clin Neurol Neurosurg* 98, 149-151 (1996)
41. Wolff, R., M. Zimmermann, G. Marquardt, H. Lanfermann, R. Nafe and V. Seifert: Glioblastoma multiforme of the brain stem in a patient with acquired immunodeficiency syndrome. *Acta Neurochir (Wien)* 144, 941-944; discussion 944-945 (2002)
42. Salvati, M., A. Frati, E. Caroli, N. Russo, F. M. Polli, M. Domenicucci and R. Delfini: Glioblastoma in kidney transplant recipients. Report of five cases. *J Neurooncol* 63, 33-37 (2003)
43. Fenstermaker, R. A. and M. J. Ciesielski: Immunotherapeutic strategies for malignant glioma. *Cancer Control* 11, 181-191 (2004)
44. Yang, L., K. Y. Ng and K. O. Lillehei: Cell-mediated immunotherapy: a new approach to the treatment of malignant glioma. *Cancer Control* 10, 138-147 (2003)
45. Ehtesham, M., K. L. Black and J. S. Yu: Recent progress in immunotherapy for malignant glioma: treatment strategies and results from clinical trials. *Cancer Control* 11, 192-207 (2004)
46. Zou, J. P., L. A. Morford, C. Chougnet, A. R. Dix, A. G. Brooks, N. Torres, J. D. Shuman, J. E. Coligan, W. H. Brooks, T. L. Roszman and G. M. Shearer: Human glioma-induced immunosuppression involves soluble factor(s) that alters monocyte cytokine profile and surface markers. *J Immunol* 162, 4882-4892 (1999)
47. Roszman, T., L. Elliott and W. Brooks: Modulation of T-cell function by gliomas. *Immunol Today* 12, 370-374 (1991)
48. Weller, M. and A. Fontana: The failure of current immunotherapy for malignant glioma. Tumor-derived TGF-beta, T-cell apoptosis, and the immune privilege of the brain. *Brain Res Brain Res Rev* 21, 128-151 (1995)
49. Akasaki, Y., G. Liu, N. H. Chung, M. Ehtesham, K. L. Black and J. S. Yu: Induction of a CD4+ T regulatory type 1 response by cyclooxygenase-2-overexpressing glioma. *J Immunol* 173, 4352-4359 (2004)
50. Chaux, P., N. Favre, B. Bonnotte, M. Moutet, M. Martin and F. Martin: Tumor-infiltrating dendritic cells are defective in their antigen-presenting function and inducible B7 expression. A role in the immune tolerance to antigenic tumors. *Adv Exp Med Biol* 417, 525-528 (1997)
51. Chaux, P., N. Favre, M. Martin and F. Martin: Tumor-infiltrating dendritic cells are defective in their antigen-presenting function and inducible B7 expression in rats. *Int J Cancer* 72, 619-624 (1997)
52. Gabrilovich, D. I., J. Corak, I. F. Ciernik, D. Kavanaugh and D. P. Carbone: Decreased antigen presentation by dendritic cells in patients with breast cancer. *Clin Cancer Res* 3, 483-490 (1997)
53. Troy, A. J., K. L. Summers, P. J. Davidson, C. H. Atkinson and D. N. Hart: Minimal recruitment and activation of dendritic cells within renal cell carcinoma. *Clin Cancer Res* 4, 585-593 (1998)
54. Yu, J. S., C. J. Wheeler, P. M. Zeltzer, H. Ying, D. N. Finger, P. K. Lee, W. H. Yong, F. Incardona, R. C. Thompson, M. S. Riedinger, W. Zhang, R. M. Prins and K. L. Black: Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. *Cancer Res* 61, 842-847 (2001)
55. Yamanaka, R., N. Yajima, T. Abe, N. Tsuchiya, J. Homma, M. Narita, M. Takahashi and R. Tanaka: Dendritic cell-based glioma immunotherapy (review). *Int J Oncol* 23, 5-15 (2003)
56. Kikuchi, T., Y. Akasaki, M. Irie, S. Homma, T. Abe and T. Ohno: Results of a phase I clinical trial of vaccination of glioma patients with fusions of dendritic and glioma cells. *Cancer Immunol Immunother* 50, 337-344 (2001)
57. Kikuchi, T., T. Abe and T. Ohno: Effects of glioma cells on maturation of dendritic cells. *J Neurooncol* 58, 125-130 (2002)
58. Yu, J. S., P. K. Lee, M. Ehtesham, K. Samoto, K. L. Black and C. J. Wheeler: Intratumoral T cell subset ratios and Fas ligand expression on brain tumor endothelium. *J Neurooncol* 64, 55-61 (2003)
59. Wintterle, S., B. Schreiner, M. Mitsdoerffer, D. Schneider, L. Chen, R. Meyermann, M. Weller and H. Wiendl: Expression of the B7-related molecule B7-H1 by glioma cells: a potential mechanism of immune paralysis. *Cancer Res* 63, 7462-7467 (2003)
60. Wiendl, H., M. Mitsdoerffer, V. Hofmeister, J. Wischhusen, A. Bornemann, R. Meyermann, E. H. Weiss, A. Melms and M. Weller: A functional role of HLA-G expression in human gliomas: an alternative strategy of immune escape. *J Immunol* 168, 4772-4780 (2002)
61. Huettner, C., W. Paulus and W. Roggendorf: Messenger RNA expression of the immunosuppressive cytokine IL-10 in human gliomas. *Am J Pathol* 146, 317-322 (1995)
62. Harada, M., K. Matsunaga, Y. Oguchi, H. Iijima, O. Ito, K. Tamada, G. Kimura and K. Nomoto: The involvement of transforming growth factor beta in the impaired antitumor T-cell response at the gut-associated lymphoid tissue (GALT). *Cancer Res* 55, 6146-6151 (1995)
63. Roncarolo, M. G., R. Bacchetta, C. Bordignon, S. Narula and M. K. Levings: Type 1 T regulatory cells. *Immunol Rev* 182, 68-79 (2001)
64. Groux, H., A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J. E. de Vries and M. G. Roncarolo: A CD4+

T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389, 737-742 (1997)
65. Chen, Y., V. K. Kuchroo, J. Inobe, D. A. Hafler and H. L. Weiner: Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 265, 1237-1240 (1994)
66. Inobe, J., A. J. Slavin, Y. Komagata, Y. Chen, L. Liu and H. L. Weiner: IL-4 is a differentiation factor for transforming growth factor-beta secreting Th3 cells and oral administration of IL-4 enhances oral tolerance in experimental allergic encephalomyelitis. *Eur J Immunol* 28, 2780-2790 (1998)
67. Levings, M. K., R. Sangregorio, C. Sartirana, A. L. Moschin, M. Battaglia, P. C. Orban and M. G. Roncarolo: Human CD25+CD4+ T suppressor cell clones produce transforming growth factor beta, but not interleukin 10, and are distinct from type 1 T regulatory cells. *J Exp Med* 196, 1335-1346 (2002)
68. Albert, M. L., M. Jegathesan and R. B. Darnell: Dendritic cell maturation is required for the cross-tolerization of CD8+ T cells. *Nat Immunol* 2, 1010-1017 (2001)
69. Steinman, R. M., S. Turley, I. Mellman and K. Inaba: The induction of tolerance by dendritic cells that have captured apoptotic cells. *J Exp Med* 191, 411-416 (2000)
70. Steinman, R. M., D. Hawiger and M. C. Nussenzweig: Tolerogenic dendritic cells. *Annu Rev Immunol* 21, 685-711 (2003)
71. Sauter, B., M. L. Albert, L. Francisco, M. Larsson, S. Somersan and N. Bhardwaj: Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. *J Exp Med* 191, 423-434 (2000)
72. Mahnke, K., E. Schmitt, L. Bonifaz, A. H. Enk and H. Jonuleit: Immature, but not inactive: the tolerogenic function of immature dendritic cells. *Immunol Cell Biol* 80, 477-483 (2002)
73. Yamazaki, S., T. Iyoda, K. Tarbell, K. Olson, K. Velinzon, K. Inaba and R. M. Steinman: Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. *J Exp Med* 198, 235-247 (2003)
74. Harizi, H., M. Juzan, V. Pitard, J. F. Moreau and N. Gualde: Cyclooxygenase-2-issued prostaglandin e(2) enhances the production of endogenous IL-10, which down-regulates dendritic cell functions. *J Immunol* 168, 2255-2263 (2002)
75. Leslie, C. C.: Properties and regulation of cytosolic phospholipase A2. *J Biol Chem* 272, 16709-16712 (1997)
76. Shono, T., P. J. Tofilon, J. M. Bruner, O. Owolabi and F. F. Lang: Cyclooxygenase-2 expression in human gliomas: prognostic significance and molecular correlations. *Cancer Res* 61, 4375-4381 (2001)
77. Joki, T., O. Heese, D. C. Nikas, L. Bello, J. Zhang, S. K. Kraeft, N. T. Seyfried, T. Abe, L. B. Chen, R. S. Carroll and P. M. Black: Expression of cyclooxygenase 2 (COX-2) in human glioma and *in vitro* inhibition by a specific COX-2 inhibitor, NS-398. *Cancer Res* 60, 4926-4931 (2000)
78. Dix, A. R., W. H. Brooks, T. L. Roszman and L. A. Morford: Immune defects observed in patients with primary malignant brain tumors. *J Neuroimmunol* 100, 216-232 (1999)
79. Dermime, S., A. Armstrong, R. E. Hawkins and P. L. Stern: Cancer vaccines and immunotherapy. *Br Med Bull* 62, 149-162 (2002)
80. Holladay, F. P., T. Heitz and G. W. Wood: Antitumor activity against established intracerebral gliomas exhibited by cytotoxic T lymphocytes, but not by lymphokine-activated killer cells. *J Neurosurg* 77, 757-762 (1992)
81. Okada, H. and I. F. Pollack: Cytokine gene therapy for malignant glioma. *Expert Opin Biol Ther* 4, 1609-1620 (2004)
82. Fong, L. and E. G. Engleman: Dendritic cells in cancer immunotherapy. *Annu Rev Immunol* 18, 245-273 (2000)
83. Yu, J. S., J. A. Burwick, G. Dranoff and X. O. Breakefield: Gene therapy for metastatic brain tumors by vaccination with granulocyte-macrophage colony-stimulating factor-transduced tumor cells. *Hum Gene Ther* 8, 1065-1072 (1997)
84. Sobol, R. E., H. Fakhrai, D. Shawler, R. Gjerset, O. Dorigo, C. Carson, T. Khaleghi, J. Koziol, T. A. Shiftan and I. Royston: Interleukin-2 gene therapy in a patient with glioblastoma. *Gene Ther* 2, 164-167 (1995)
85. Joki, T., T. Kikuchi, Y. Akasaki, S. Saitoh, T. Abe and T. Ohno: Induction of effective antitumor immunity in a mouse brain tumor model using B7-1 (CD80) and intercellular adhesive molecule 1 (ICAM-1; CD54) transfection and recombinant interleukin 12. *Int J Cancer* 82, 714-720 (1999)
86. Soos, J. M., J. I. Krieger, O. Stuve, C. L. King, J. C. Patarroyo, K. Aldape, K. Wosik, A. J. Slavin, P. A. Nelson, J. P. Antel and S. S. Zamvil: Malignant glioma cells use MHC class II transactivator (CIITA) promoters III and IV to direct IFN-gamma-inducible CIITA expression and can function as nonprofessional antigen presenting cells in endocytic processing and CD4(+) T-cell activation. *Glia* 36, 391-405 (2001)
87. Banchereau, J. and R. M. Steinman: Dendritic cells and the control of immunity. *Nature* 392, 245-252 (1998)
88. Heimberger, A. B., L. E. Crotty, G. E. Archer, R. E. McLendon, A. Friedman, G. Dranoff, D. D. Bigner and J. H. Sampson: Bone marrow-derived dendritic cells pulsed with tumor homogenate induce immunity against syngeneic intracerebral glioma. *J Neuroimmunol* 103, 16-25 (2000)
89. Yamanaka, R., S. A. Zullo, R. Tanaka, M. Blaese and K. G. Xanthopoulos: Enhancement of antitumor immune response in glioma models in mice by genetically modified dendritic cells pulsed with Semliki forest virus-mediated complementary DNA. *J Neurosurg* 94, 474-481 (2001)
90. Akasaki, Y., T. Kikuchi, S. Homma, T. Abe, D. Kofe and T. Ohno: Antitumor effect of immunizations with fusions of dendritic and glioma cells in a mouse brain tumor model. *J Immunother* 24, 106-113 (2001)
91. Witham, T. F., M. L. Erff, H. Okada, W. H. Chambers and I. F. Pollack: 7-Hydroxystaurosporine-induced apoptosis in 9L glioma cells provides an effective antigen source for dendritic cells and yields a potent vaccine strategy in an intracranial glioma model. *Neurosurgery* 50, 1327-1334; discussion 1334-1325 (2002)
92. Yamanaka, R., N. Yajima, N. Tsuchiya, J. Honma, R. Tanaka, J. Ramsey, M. Blaese and K. G. Xanthopoulos: Administration of interleukin-12 and -18 enhancing the antitumor immunity of genetically modified dendritic cells that had been pulsed with Semliki forest virus-mediated

tumor complementary DNA. *J Neurosurg* 97, 1184-1190 (2002)
93. Saito, R., M. Mizuno, N. Nakahara, T. Tsuno, T. Kumabe, T. Yoshimoto and J. Yoshida: Vaccination with tumor cell lysate-pulsed dendritic cells augments the effect of IFN-beta gene therapy for malignant glioma in an experimental mouse intracranial glioma. *Int J Cancer* 111, 777-782 (2004)
94. Mayordomo, J. I., T. Zorina, W. J. Storkus, L. Zitvogel, C. Celluzzi, L. D. Falo, C. J. Melief, S. T. Ildstad, W. M. Kast, A. B. Deleo and *et al.*: Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. *Nat Med* 1, 1297-1302 (1995)
95. Hung, K., R. Hayashi, A. Lafond-Walker, C. Lowenstein, D. Pardoll and H. Levitsky: The central role of CD4(+) T cells in the antitumor immune response. *J Exp Med* 188, 2357-2368 (1998)
96. Bourgeois, C., B. Rocha and C. Tanchot: A role for CD40 expression on CD8+ T cells in the generation of CD8+ T cell memory. *Science* 297, 2060-2063 (2002)
97. Bennett, S. R., F. R. Carbone, F. Karamalis, R. A. Flavell, J. F. Miller and W. R. Heath: Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* 393, 478-480 (1998)
98. Ridge, J. P., F. Di Rosa and P. Matzinger: A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. *Nature* 393, 474-478 (1998)
99. Schoenberger, S. P., R. E. Toes, E. I. van der Voort, R. Offringa and C. J. Melief: T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature* 393, 480-483 (1998)
100. Tatsumi, T., L. S. Kierstead, E. Ranieri, L. Gesualdo, F. P. Schena, J. H. Finke, R. M. Bukowski, J. Mueller-Berghaus, J. M. Kirkwood, W. W. Kwok and W. J. Storkus: Disease-associated bias in T helper type 1 (Th1)/Th2 CD4(+) T cell responses against MAGE-6 in HLA-DRB10401(+) patients with renal cell carcinoma or melanoma. *J Exp Med* 196, 619-628 (2002)
101. Slager, E. H., M. Borghi, C. E. van der Minne, C. A. Aarnoudse, M. J. Havenga, P. I. Schrier, S. Osanto and M. Griffioen: CD4+ Th2 cell recognition of HLA-DR-restricted epitopes derived from CAMEL: a tumor antigen translated in an alternative open reading frame. *J Immunol* 170, 1490-1497 (2003)
102. Gately, M. K., L. M. Renzetti, J. Magram, A. S. Stern, L. Adorini, U. Gubler and D. H. Presky: The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu Rev Immunol* 16, 495-521 (1998)
103. Nakanishi, K., T. Yoshimoto, H. Tsutsui and H. Okamura: Interleukin-18 regulates both Th1 and Th2 responses. *Annu Rev Immunol* 19, 423-474 (2001)
104. Trinchieri, G., S. Pflanz and R. A. Kastelein: The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. *Immunity* 19, 641-644 (2003)
105. Lankford, C. S. and D. M. Frucht: A unique role for IL-23 in promoting cellular immunity. *J Leukoc Biol* 73, 49-56 (2003)
106. Pirhonen, J., S. Matikainen and I. Julkunen: Regulation of virus-induced IL-12 and IL-23 expression in human macrophages. *J Immunol* 169, 5673-5678 (2002)
107. Belladonna, M. L., J. C. Renauld, R. Bianchi, C. Vacca, F. Fallarino, C. Orabona, M. C. Fioretti, U. Grohmann and P. Puccetti: IL-23 and IL-12 have overlapping, but distinct, effects on murine dendritic cells. *J Immunol* 168, 5448-5454 (2002)
108. Liu, G., H. Ng, Y. Akasaki, X. Yuan, M. Ehtesham, D. Yin, K. L. Black and J. S. Yu: Small interference RNA modulation of IL-10 in human monocyte-derived dendritic cells enhances the Th1 response. *Eur J Immunol* 34, 1680-1687 (2004)
109. Moore, K. W., R. de Waal Malefyt, R. L. Coffman and A. O'Garra: Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 19, 683-765 (2001)
110. Yu, J. S., G. Liu, H. Ying, W. H. Yong, K. L. Black and C. J. Wheeler: Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. *Cancer Res* 64, 4973-4979 (2004)
111. Kikuchi, T., Y. Akasaki, T. Abe, T. Fukuda, H. Saotome, J. L. Ryan, D. W. Kufe and T. Ohno: Vaccination of glioma patients with fusions of dendritic and glioma cells and recombinant human interleukin 12. *J Immunother* 27, 452-459 (2004)
112. Kikuchi, T., Y. Akasaki, T. Abe and T. Ohno: Intratumoral injection of dendritic and irradiated glioma cells induces anti-tumor effects in a mouse brain tumor model. *Cancer Immunol Immunother* 51, 424-430 (2002)
113. Ehtesham, M., P. Kabos, M. A. Gutierrez, K. Samoto, K. L. Black and J. S. Yu: Intratumoral dendritic cell vaccination elicits potent tumoricidal immunity against malignant glioma in rats. *J Immunother* 26, 107-116 (2003)
114. Inaba, K., M. Inaba, M. Naito and R. M. Steinman: Dendritic cell progenitors phagocytose particulates, including bacillus Calmette-Guerin organisms, and sensitize mice to mycobacterial antigens *in vivo*. *J Exp Med* 178, 479-488 (1993)
115. Yamanaka, R., T. Abe, N. Yajima, N. Tsuchiya, J. Homma, T. Kobayashi, M. Narita, M. Takahashi and R. Tanaka: Vaccination of recurrent glioma patients with tumour lysate-pulsed dendritic cells elicits immune responses: results of a clinical phase I/II trial. *Br J Cancer* 89, 1172-1179 (2003)
116. Wheeler, C. J., A. Das, G. Liu, J. S. Yu and K. L. Black: Clinical responsiveness of glioblastoma multiforme to chemotherapy after vaccination. *Clin Cancer Res* 10, 5316-5326 (2004)
117. Chu, W., B. J. Pak, M. R. Bani, M. Kapoor, S. J. Lu, A. Tamir, R. S. Kerbel and Y. Ben-David: Tyrosinase-related protein 2 as a mediator of melanoma specific resistance to cis-diamminedichloroplatinum(II): therapeutic implications. *Oncogene* 19, 395-402 (2000)
118. Liu, G.: Cytotoxic T cell targeting of TRP-2 sensitizes human malignant glioma to chemotherapy. *Oncogene* (2005)

Key Words: Dendritic Cell, T Cell, Central Nervous System, Glioma, Cancer, Glial cell, Immunotherapy, Tolerance **,** Review

Send correspondence to: Dr John S. Yu, Maxine Dunitz Neurosurgical Institute, Cedars-Sinai Medical Center, Suite 800 East, 8631 West 3rd Street, Los Angeles, California 90048, Tel: 310-423-0875, Fax: 310-423-0810, E-mail: yuj@cshs.org

http://www.bioscience.org/current/vol10.htm